melted at 158–160° and was identical with the product (XII) secured via NBS oxidation of XI.

(B) **Alkaline Hydrolysis and Acetylation.**—To a solution of 400 mg. of the 21-bromotrione XVI in 60 cc. of methanol and 30 cc. of water, 6.3 cc. of a 3.7% methanolic potassium hydroxide solution was added under nitrogen. The solution which immediately turned yellow, was held at room temperature for 8 minutes and then acidified with acetic acid. The solution was then taken to dryness *in vacuo*, extracted with methylene chloride, washed with water and evaporated to dryness. The residue was warmed on the steam-bath for a few minutes with 1 cc. of acetic anhydride and 2 cc. of pyridine and then let stand for 0.5 hr. Working up in the usual manner and crystallizing from ether–petroleum ether afforded 215 mg. of substance, m.p. 148–152°, which after recrystallization melted at 158–160° and proved identical with XII secured from the NBS oxidation.

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH AND DEVELOPMENT DIVISION OF THE SCHERING CORPORATION]

Steroidal Amines. III. 16α-Amino-Substituted Pregnanes[1]

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RECEIVED JANUARY 13, 1956

5,16-Pregnadien-3β-ol-20-one (II) and its acetate were treated with unhindered primary and secondary amines in the presence of a basic catalyst. The products are substituted 16α-amino-5-pregnen-3β-ol-20-ones, some of which were also reduced to the corresponding 5-pregnene-3β,20-diols and allopregnanediols. The derivatives most closely resembling steroidal alkaloids such as rubijervine (I) do, in fact, have pharmacological properties similar to these alkaloids.

In the process of saponification of 5,16-pregnadien-3β-ol-20-one acetate with methanolic potassium hydroxide, the major product was not pregnadienolone but 16α-methoxy-5-pregnen-3β-ol-20-one.[2] It was thus apparent that the Δ^{16}-20-ketone system is favorable for base-catalyzed additions to the 16-carbon of nucleophilic reagents with an active hydrogen.

Marker had observed[3a] that Grignard reagents attacked pregnadienolone acetate at both the 16-carbon and the 20-carbonyl. With the more hindered reagents, the only isolable products were 16-alkyl-pregnenolones. Similarly the nucleophilic carbon of diazomethane attacks the 16-position.[3b]

Recently, Romo, *et al.*,[4] found confirmation of this indication in the addition of benzylmercaptan to give 16-thiobenzyl derivatives.

In an approach to synthetics similar to the various steroidal alkaloids, *e.g.*, rubijervine[5] (I), having an amino substituent at C_{16}, we investigated the addition of various amino compounds to 5,16-pregnadienolone (II) and its acetate, usually using basic catalysis.[6] When steric factors were not involved, the reaction proceeded readily and, under the proper conditions, more or less to completion, to give 16α-aminopregnenolones (III). The preferred conditions varied somewhat for different amines, apparently depending on their water solubility and base strength. The catalysts used were aqueous alkali, quaternary ammonium hydroxides and quaternary ammonium resin bases. In a typical reaction the amine with solvent was warmed to dissolve the steroid and stirred at room temperature overnight in the presence of the catalyst. When amines were used without catalysis, the reaction either did not occur or was less than 25% complete. The use of at least one mole of base permitted concomitant saponification of the 3-acetate, but a greater excess did not make any further difference.

It was found that primary alkyl and cycloalkyl amines added in the presence of strong base, while aniline did not add readily. Of the secondary amines, however, only dimethylamine and the cyclic amines, pyrrolidine, piperidine, morpholine and β- and γ-pipecoline added. Diethylamine reacted only slightly with the pregnadienolone as shown by the decreased ultraviolet absorption, but the product was not isolated. Similarly, α-pipecoline did not react well. It was apparent that there are notable steric limitations imposed on amines which can add. In addition to secondary alkylamines, it was found that primary amines attached to a tertiary carbon, *e.g.*, *t*-butylamine, would not add. Furthermore, primary amines on a secondary carbon added with difficulty or only partially. This was particularly so when the resin base was used as catalyst. Thus *sec*-butylamine would add only with the non-polymeric catalysts. The extent of reaction in the crude product was easily determined by an examination of the remaining ultraviolet absorption due to the α,β-unsaturated carbonyl of the unreacted starting material. The properties of the 16α-aminopregnenolones prepared are given in Table II. Many products crystallized as solvates and frequently the solvent could not be removed completely.

Since they could not be prepared directly by addition, some quaternary amines were prepared by alkylation of the corresponding secondary and tertiary amino steroids. Thus 16-benzylaminopregnenolone was converted to the benzyl dimethylammonium iodide with methyl iodide and sodium hydroxide, and the methylpiperidinium

(1) Presented in part before the 128th Meeting of the American Chemical Society, Minneapolis, Minn., Sept. 15, 1955. Paper I, see ref. 6; paper II, H. L. Herzog, C. Payne and E. B. Hershberg, THIS JOURNAL, **77**, 5324 (1955).

(2) (a) D. K. Fukushima and T. F. Gallagher, *ibid.*, **73**, 196 (1951); (b) D. Gould, F. Gruen and E. B. Hershberg, *ibid.*, **75**, 2510 (1953).

(3) (a) R. E. Marker and H. M. Crooks, Jr., *ibid.*, **64**, 1280 (1942); (b) A. Wettstein, *Helv. Chim. Acta*, **27**, 1803 (1944); A. Sandoval, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 2383 (1951).

(4) J. Romo, M. Romero, C. Djerassi and G. Rosenkranz, *ibid.*, **73**, 1528 (1951).

(5) Y. Sato and W. A. Jacobs, *J. Biol. Chem.*, **179**, 623 (1949).

(6) A preliminary announcement by D. Gould, E. L. Shapiro and E. B. Hershberg appeared in THIS JOURNAL, **76**, 5567 (1954).

TABLE I

MOLECULAR ROTATION DIFFERENCES IN 16-SUBSTITUTED STEROIDS

Compound	$[\alpha]_D$	M_D	ΔM_D (16H − 16R)
Allopregnane-3β,20α-diol diacetate[a]	+ 2°	+ 8	...
16α-Acetoxy-[a]	−58	−268	−276
16β-Acetoxy-[b]	+25	+116	+108
Allopregnane-3β,20β-diol diacetate[a]	+32	+125	...
16α-Acetoxy-[b]	−25	−208	−333
16β-Acetoxy-[b]	+48	+222	+ 97
Estradiol-3,17β[c]	+81	+220	...
16β-Hydroxy-[d]	+61	+176	− 44
16β-Hydroxy-[e]	+88	+253	+ 33
Androstane-3β,17β-diol[f]	+ 4.2	+ 12	...
16α-Hydroxy-[g]	−19	− 59	− 71
16β-Hydroxy-[h]	+18	+ 55	+ 43
5-Pregnen-3β-ol-20-one[i]	+28	+ 89	...
16α-Methoxy-[2b]	−24.4	− 81	−170
16α-Methylamino-	−22.6	− 78	−167
5-Pregnen-3β-ol-20-one acetate[j]	+18.9	+ 68	...
16α-Methoxy-[2]	−28.2	−110	−178
16α-Piperidino-	−22.0	− 97	−165
Allopregnan-3β-ol-20-one[k]	+98	+312	...
16α-Piperidino-	+49.6	+198	−114

[a] H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **184**, 259 (1950). [b] H. Hirschmann, F. B. Hirschmann and M. A. Daus, *ibid.*, **178**, 751 (1949). [c] B. Whitman, O. Wintersteiner and E. Schwenk, *ibid.*, **118**, 789 (1937). [d] S. Thayer, L. Levin and E. Doisy, *ibid.*, **91**, 655 (1931). [e] M. Hoffman and H. Darby, THIS JOURNAL, 66, 150 (1944). [f] A. Butenandt, K. Tscherning and G. Hanisch, *Ber.*, **68**, 2097 (1935). [g] L. Ruzicka, V. Prelog and P. Wieland, *Helv. Chim. Acta*, **28**, 1609 (1945). [h] M. Hoffman and M. Lott, THIS JOURNAL, **71**, 719 (1949). [i] A. Butenandt and G. Fleischer, *Ber.*, **70**, 96 (1937). [j] D. Gould and W. Tarpley, *Science*, **113**, 417 (1951). [k] A. Butenandt and L. Mamoli, *Ber.*, **68**, 1847 (1935).

TABLE II

16α-AMINO-5-PREGNEN-3β-OL-20-ONES

16-Substituent	M.p., °C.	$[\alpha]^{25}D$	Formula	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Solvent[d]
Methylamino	166.5–167.5	−22.6	$C_{22}H_{35}O_2N$	76.47	10.21	4.06	76.56	10.22	4.01	D
Ethylamino	113.4–115.4	−23.2	$C_{23}H_{37}O_2N\cdot(CH_3)_2CO$	74.95	10.16	3.36	74.75	9.98	3.24	D
n-Propylamino	85–88	−21.8	$C_{24}H_{39}O_2N$	77.16	10.52	3.75	77.65	10.85	3.55	C–F
n-Propylamino	84–87.5	−19.9[a]	$C_{24}H_{39}O_2N\cdot C_6H_6$	79.77	10.04	3.10	79.62	9.71	3.29	C
iso-Propylamino	133–135	−26.4	$C_{24}H_{39}O_2N$	77.16	10.52	3.75	76.79	10.32	3.69	D–F
Allylamino·HCl	247–248.5 d.	+10.5	$C_{24}H_{37}O_2N\cdot HCl$	(Cl, 8.69)		3.43	(Cl, 9.02)		3.27	B–D
n-Butylamino	106–108	−25.0	$C_{25}H_{41}O_2N$	77.47	10.66	3.61	77.82	10.40	3.58	C
iso-Butylamino	108–110	−27.6	$C_{25}H_{41}O_2N$[c]	77.47	10.66	3.61[c]	77.97	10.36	3.20	C
sec-Butylamino	120–122	−32.2	$C_{25}H_{41}O_2N$	77.47	10.66	3.61	77.71	10.42	3.40	C–F
n-Amylamino	124.5–126.0	−20.6	$C_{26}H_{43}O_2N$	77.75	10.79	3.49	77.56	10.83	3.35	C–F
iso-Amylamino	130–133	−23.6	$C_{26}H_{43}O_2N$	77.75	10.79	3.49	77.55	10.91	3.36	F–C
n-Hexylamino	111.4–112.6	−23.3	$C_{27}H_{45}O_2N$	78.02	10.91	3.37	78.31	11.11	3.39	D
iso-Hexylamino	124–126	−18.1	$C_{27}H_{45}O_2N$	78.02	10.91	3.37	78.24	10.96	3.23	D
iso-Hexylamino·HCl	251–252 d.	+ 9.3	$C_{27}H_{45}O_2N\cdot HCl$	(Cl, 7.84)		3.10	(Cl, 7.69)		3.29	B–D
2-Ethylhexylamino	102.5–105.0	−20.6	$C_{29}H_{49}O_2N$	78.50	11.13	3.16	78.71	10.90	2.95	C–F
Benzylamino	148–150	−38.8	$C_{28}H_{39}O_2N$	79.76	9.32	3.32	79.49	9.32	3.34	C–D
Dimethylamino	204.0–205.5	−19.0	$C_{23}H_{37}O_2N$	76.83	10.37	3.90	76.97	10.46	3.73	C
Pyrrolidino	145–147	−17.6	$C_{25}H_{39}O_2N$[b]	77.87	10.20	3.63[b]	76.43	9.85	3.36	C, E
Morpholino	180–181	−11.8	$C_{25}H_{39}O_3N$	74.77	9.79	3.49	74.45	9.94	3.40	C

[a] $[\alpha]^{25}D$ −0.1 (EtOH); −14.8 ($CHCl_3$). [b] Calcd. for $C_{25}H_{39}O_2N + {}^1/_4CH_3COOC_2H_5$: C, 76.61; H, 10.14; N, 3.44. [c] Calcd. for $C_{25}H_{41}O_2N + {}^1/_4C_6H_6$: C, 78.18; H, 10.52; N, 3.44. [d] Crystallization solvent: A, ethanol; B, methanol; C, benzene; D, acetone; E, ethyl acetate; F, hexane.

derivative was prepared by treatment of 16α-piperidinopregnenolone with methyl iodide.

The majority of the products were relatively unstable. They could not be safely heated over 60°, and decomposed with acid or base, particularly in the presence of an alcohol. Thus 16-piperidinopregnenolone as the hydrochloride, acetate salt or free base in dilute alcohol tended to revert to 5,16-pregnadien-3β-ol-20-one on standing at room temperature. Also distillation of the reaction mixture to remove excess amine, or concentration of a methanol solution, led to decomposition.

Owing to the relative instability of the products, it was necessary to use fairly gentle conditions to

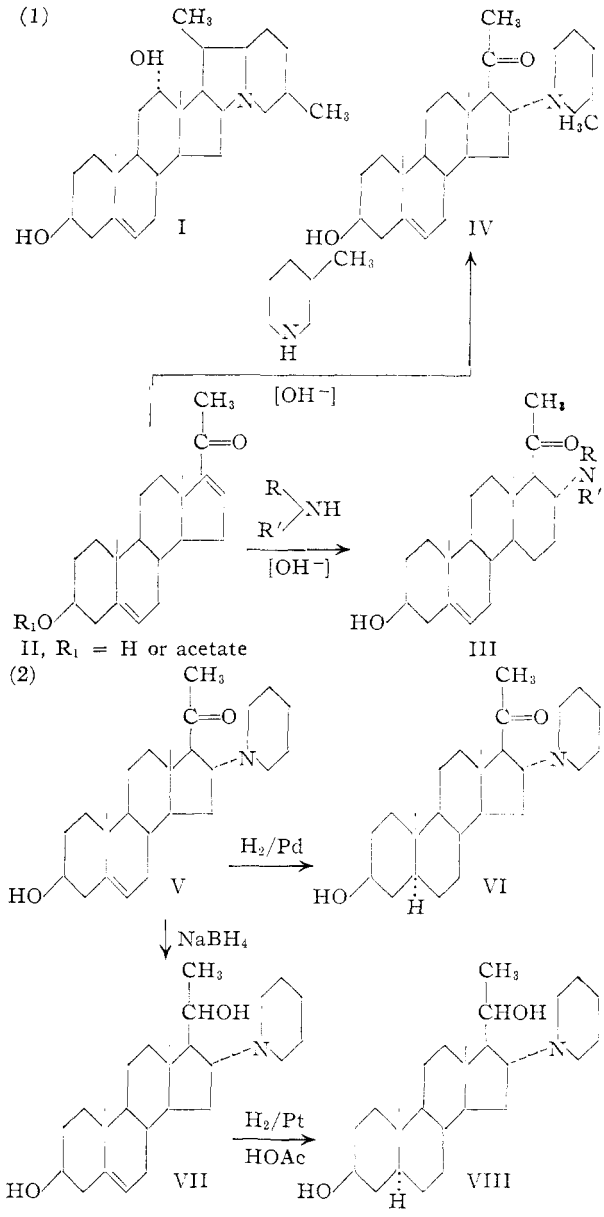
prepare 3-esters, several of which were prepared from 16α-piperidinopregnenolone. The acid anhydrides or chlorides in pyridine at room temperature were generally satisfactory. In the case of the 3-veratrate, the esterification proceeded too slowly at room temperature and had to be warmed. This led to some deamination, and heating as long as 24 hours led to complete deamination. Indeed, it was possible to prepare highly purified esters from a technical grade of 5,16-pregnadienolone, through conversion to 16-amines and subsequent deamination, usually by heating in the desired acid anhydride.

The configuration of the amino substituent at the 16-position has been assumed to be α, or *trans* to the side chain at C-17. This hypothesis appears to be entirely reasonable in the light of the available evidence. Thus, Fukushima and Gallagher[2a] have deduced from rotational and steric evidence that the base-catalyzed addition of alcohols to 5,16-pregnadienolone leads to 16α-alkoxy-pregnenolones. Hirschmann and co-workers[7] have furthermore shown conclusively that benzyl alcohol adds in the α-configuration, since they obtained a known 16α-hydroxy steroid upon reductive removal of the benzyl group. Rotational evidence also supports the α-configuration assigned in the case of the 16-aminopregnanes, as illustrated in Table I.

All known 16β-substituents have a positive rotatory contribution while all known 16α-substituents have a negative contribution. The 16-aminopregnenolones all have negative rotations with ΔM_D −180 (mean). The close approximation between 16α-methoxy-5-pregnen-3β-ol-20-one (ΔM_D −170) and the 16-methylaminopregnenolone (ΔM_D −167) is further confirmation that this amine, and the others, are α-oriented. Even in the 5α-series, 16α-piperidinoallopregnan-3β-ol-20-one, although having a positive M_D +198, has a negative ΔM_D −114 which placed it with the 16α-substituted steroids.

When piperidine or β-pipecoline was added, compounds V and IV, respectively, were obtained. These resemble rubijervine (I) on paper but, of course, differ considerably in their stereoconfiguration, since the C_{16}–N linkage is α in V and IV. Conversely, the C_{16}–N bond must be β in the natural steroidal alkaloids to allow for the *cis*-fusion of the D and E rings, and to conform with the known β-configuration of the 17-side chain.[8]

In spite of this difference, 16α-piperidino-5-pregnen-3β-ol-20-one (V) and 16α-(3′-methylpiperidino)-5-pregnen-3β-ol-20-one (IV) showed pharmacological activity in many respects similar to the steroidal tertiary alkamines of the veratrum group such as rubijervine. Thus a hypotensive effect of the order of rubijervine (I)[9] has been demonstrated at 1–2 mg./kg. in dogs,[10] without,



however, showing respiratory depression.

Surprisingly, these compounds also show the actions of the secondary steroidal alkamines such as jervine and veratramine.[11] Previous to this discovery only those steroidal alkaloids with a secondary amine exhibited bradycrotic activity including the ability to counteract specifically the cardio-accelerating effect of epinephrine without inhibiting its inotropic action.[12,13] These syn-

(7) H. Hirschmann, F. B. Hirschmann and M. A. Daus, THIS JOURNAL, **74**, 539 (1952).

(8) See the work of L. C. Craig, W. A. Jacobs, F. C. Uhle and Y. Sato reviewed in L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publishing Corp., New York, N. Y., 1949.

(9) G. L. Maison, E. Gotz and J. W. Stutzman, *J. Pharmacol. Exptl. Therap.*, **103**, 74 (1951).

(10) We are indebted to Dr. O. Krayer, Department of Pharmacology, Harvard Medical School, for his continued interest and advice, and to Drs. S. Margolin and G. Lu, Pharmacology Department, Schering Corporation, for the pharmacological results.

(11) For structure see J. Fried, O. Wintersteiner, M. Moore, B. M. Iselin and A. Klingsberg, THIS JOURNAL, **73**, 2970 (1951); C. Tamm and O. Wintersteiner, *ibid.*, **74**, 3842 (1952); O. Wintersteiner and N. Hosansky, *ibid.*, **74**, 4474 (1952).

(12) For statements of this hypothesis and pharmacology of secondary alkaloids, see O. Krayer, *J. Pharmacol. Exptl. Therap.*, **96**, 422 (1949); *Arch. f. exper. Path. u. Pharmakol.*, **209**, 405 (1950); O. Krayer, F. C. Uhle and P. Ourisson, *J. Pharmacol. Exptl. Therap.*, **102**, 261 (1951).

(13) The non-steroidal tertiary amino alkaloids, quinine and quinidine, are reported to have antiaccelerator activity at high dosage; see O. Krayer, *J. Pharmacol. Exptl. Therap.*, **100**, 146 (1950).

TABLE III

DERIVATIVES OF 16α-PIPERIDINOALLOPREGNAN-3β-OL

Derivative	M.p., °C.	$[\alpha]^{25}D$	Formula	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Solvent[d]
Δ^5-20-Keto[a]	149–151	−23.5°	$C_{26}H_{41}O_2N$	78.14	10.34	3.51	78.28	10.22	3.60	B, C
Δ^5-20-Keto[a]	160–162	−24.7	$C_{26}H_{41}O_2N$	78.14	10.34	3.51	78.17	10.14	3.51	C
Δ^5-20-Keto·HCl	240–242 d.	+ 8.7	$C_{26}H_{41}O_2N$·HCl	(Cl, 8.13)		3.22	(Cl, 8.06)		3.21	B–D
Δ^5-20-Keto·Acid Maleate	203–205 d.	+ 8.4	$C_{30}H_{45}O_6N$	69.87	8.80	2.72	70.27	8.69	2.77	B
Δ^5-20-Keto·Bitartrate	166,[b] 203–5 d.	+17.1[b]	$C_{30}H_{47}O_8N$			2.55			2.72	A
Δ^5-20-Keto-3-acetate	176–178	−22.0	$C_{28}H_{43}O_3N$	76.14	9.81	3.17	75.95	10.03	3.43	A
Δ^5-20-Keto-3-acetate·HCl	228–230 d.	+ 5.2	$C_{28}H_{43}O_3N$·HCl	(Cl, 7.42)		2.93	(Cl, 7.57)		2.80	C
Δ^5-20-Keto-3-*i*-Butyrate	140–141	−12.6	$C_{30}H_{47}O_3N$	76.71	10.09	2.98	76.79	10.10	3.27	A
Δ^5-20-Keto-3-*i*-Butyrate·HCl	238 d.	+ 0	$C_{30}H_{47}O_3N$·HCl	(Cl, 7.01)		2.77	(Cl, 6.92)		3.04	C
Δ^5-20-Keto-3-veratrate	154–155.5	+18.6	$C_{35}H_{49}O_5N$	74.56	8.76	2.48	74.57	9.09	2.43	A
Δ^5-20-Keto-3-veratrate·HCl	243.5–244.5 d.	+24.6	$C_{35}H_{49}O_5N$·HCl	(Cl, 5.91)		2.33	(Cl, 5.76)		2.57	C
Δ^5-20-Keto-3′-methyl	172–175	−23.2	$C_{27}H_{43}O_2N$	78.40	10.48	3.39	78.49	10.21	3.52	C–F
Δ^5-20-Keto-3′-methyl·HCl[c]	215–217.5 d.	+16.7	$C_{27}H_{43}O_2N$·HCl	(Cl, 7.88)		3.11	(Cl, 7.61)		3.09	B–D
Δ^5-20-Keto-3′-methyl·HCl[c]	222–226.5 d.	+ 8.8	$C_{27}H_{43}O_2N$·HCl	(Cl, 7.88)		3.11	(Cl, 7.72)		3.40	B–D
Δ^5-20-Hydroxy	184–186	−97.6	$C_{26}H_{43}O_2N$	77.75	10.79	3.49	77.51	10.90	3.34	A, B
Δ^5-20-Hydroxy·HCl	292.5–293 d.	−43.7	$C_{26}H_{43}O_2N$·HCl	(Cl, 8.09)		3.20	(Cl, 7.92)		3.48	B
20-Keto	169.5–170.5	+49.6	$C_{26}H_{43}O_2N$	77.75	10.79	3.49	77.87	10.59	3.43	A
20-Hydroxy[a]	178–180	−55.2	$C_{26}H_{45}O_2N$	77.36	11.24	3.47	77.40	10.90	3.22	C, D
20-Hydroxy[a]	185–190	−57.0	$C_{26}H_{45}O_2N$	77.36	11.24	3.47	77.11	10.90	3.16	D
Δ^5-20-Keto-4′-methyl	168–170	−23.8	$C_{27}H_{43}O_2N$	78.40	10.48	3.39	78.10	10.46	3.46	C

[a] Two forms. [b] Resolidifies at 175°, $[\alpha]^{25}D$ +15.4° (H_2O). [c] Two 3′-methyl isomers. [d] Crystallization solvent: A, ethanol; B, methanol; C, benzene; D, acetone; E, ethyl acetate; F, hexane.

thetic tertiary alkamines nevertheless have anti-accelerator properties of the order of activity of jervine[14] when used in anesthetized dogs and isolated heart preparations.[15]

In further agreement with the known pharmacological properties of natural secondary alkamines, these tertiary amines stimulate the central nervous system in such a manner as to cause severe convulsions in non-anesthetized dogs at anti-accelerator doses. For this reason, various derivatives listed in Table III were prepared by esterification or by selective reduction as outlined in scheme (2) (V to VI, VII and VIII) to give the 5-allo-dihydro and the 20-hydroxyl derivatives. The change from a 20-keto to a 20-hydroxyl group did not seriously alter the pharmacological effects, but esterification reduced the activity.

Experimental[16]

Preparation of 16α-Aminopregnenolones.—The reactions were generally carried out using 4–10 parts of the starting amine as solvent, particularly with water-soluble amines. When pregnadienolone acetate was used, rather than pregnadienolone, it was necessary to heat for 1–2 hr. to saponify the ester. Continued heating, however, prevented completion of the addition reaction, which was therefore carried out at room temperature. Usually, water-precipitation was satisfactory for isolation of the product. When the excess starting amine was not soluble, it was useful to adjust the *p*H to *ca.* 6.5 with acetic acid which dissolved the amine but not the steroid. The process of crystallization of the crude products was complicated by solvation of the steroids except when the melting point was at least 180°. Benzene was used most often, although gels formed frequently. By careful manipulation, crystalline products were obtained, still containing solvent of crystallization which could be removed only with difficulty or incompletely. Other solvents, which were no better in this respect, included ethyl acetate, acetone, acetone–ether, acetone–hexane, benzene–hexane, methanol and ethanol. A typical procedure is given below.

Variations. (1) **Solvent.**—The starting amine was usually satisfactory as a solvent, particularly in amounts of about 10 volumes. When such an excess was not available or did not readily dissolve the steroid, diluents were necessary. Convenient solvents include dioxane, tetrahydrofuran, pyridine and *t*-butyl alcohol. These solvents permit water-precipitation as a means of isolation of the product.

(2) **Catalyst.**—The most generally useful catalyst was 25–50% aqueous alkali (usually potassium hydroxide) in amounts equivalent to about 0.1–0.25 mole in reactions which did not involve saponification, and equivalent to 1.25–1.5 moles where saponification of a single ester was required. Other bases used were benzyltrimethylammonium hydroxide in water or methanol and choline in water, in the same equivalencies stated above.[17]

All of the catalysts thus far mentioned gave a second liquid phase, and therefore required very efficient stirring to promote action. The most convenient catalyst, however, was an anion exchange resin (hydroxide form),[18] which was used in an amount equal to the weight of steroid. In this case the degree of dispersion no longer was critical and the catalyst was readily removed by filtration after the reaction. The more hindered amines, however, required

(14) O. Krayer and L. H. Briggs, *Brit. J. Pharm. Chem.*, **5**, 118, 517 (1950).

(15) A preliminary announcement of the pharmacology was published by S. Margolin, G. Lu, J. Yelnoski and A. Makovsky, *Science*, **120**, 576 (1954).

(16) All melting points are corrected. Analyses and optical data were obtained by the Microanalytical and Physical Chemical Departments of this Laboratory. Rotations of amines are in dioxane and salts are in 95% ethanol unless stated differently.

(17) It is necessary to keep the amount of methanol to a minimum since base catalyzes the formation of 16α-methoxy-5-pregnen-3β-ol-20-one (see note 2). Isopropyl alcohol or *t*-butyl alcohol solutions may be used, however, since the extent of their addition is negligible.

(18) Amberlite IRA-400, 410 and XE-75, 98 (Rohm and Haas).

several days reaction time, and *sec*-butylamine did not add to a useful extent. Saponification occurred as before when using the other catalysts.

Salts.—Hydrochlorides were prepared in the usual manner by treatment of benzene solutions with ethereal hydrogen chloride; or by treatment of methanol or acetone solutions with concentrated hydrochloric acid. Salts of organic acids were prepared by treatment of acetone or alcohol solutions of the amine with aqueous or alcoholic solutions of the acid. Attempts to prepare bis-salts of dicarboxylic acids led to crude monobasic salts.

The bitartrate of 16α-piperidinopregnenolone was shown to have a free carboxyl group by titration of an aqueous solution using sodium hydroxide and phenolphthalein. At one equivalent (*p*H 6.4), a precipitate formed, but the indicator did not change till two equivalents of base had been added. The precipitate proved to be the free steroid amine.

In working up incomplete reactions, it was most convenient to dissolve the steroid amine in dilute hydrochloric or aqueous acetic acid, filter off the unreacted starting material and reprecipitate the steroid amine by making the filtrate basic. The hydrochlorides of 16α-piperidino-5-pregnen-3β-ol-20-one 3-esters, however, were rather poorly soluble in water and surprisingly soluble in benzene, from which they were crystallized. In some cases they were even soluble in ether.

16α-Piperidino-5-pregnen-3β-ol-20-one.—One gram of 5,16-pregnadien-3β-ol-20-one acetate was dissolved by warming in 4 ml. of piperidine and treated with 0.25 g. of 86% potassium hydroxide in 0.3 ml. of water. The two-phase mixture was stirred while heating on the steam-bath for 2 hr., and then at room temperature overnight. The reaction mixture was poured into 400 ml. of water while stirring. After standing 1 hr., the precipitate was collected by filtration, washed well with water and dried *in vacuo* over phosphorus pentoxide, wt. 1.15 g., m.p. 131–135°. At 238 mμ, ε 0 (EtOH) indicating that no starting material remained.

Crystallization from benzene gave needles, m.p. 146–148°. A sample was crystallized to give the pure benzene-solvated product, m.p. 149–151°. The rotation was $[\alpha]^{25}D$ −20.0° and the infrared spectrum (CS_2 solution) showed the following peaks: 2.79 μ (OH), 3.00 (bonded OH), 5.14 and 5.54 (benzene), 5.88 (saturated C_{20}-carbonyl).

Upon drying over toluene at 1 mm. for 6 hr. (necessary to remove the benzene), the product had m.p. 149–151°, with slight sintering at 131–138°, $[\alpha]^{25}D$ −23.5, $[\alpha]^{25}D$ ±0° (EtOH). The infrared spectrum (CS_2) still showed peaks at 2.79, 3.00 and 5.88 μ with the benzene peaks at 5.14 and 5.54 μ missing. In Nujol (mull), the peaks were at 2.94, 3.17 and 5.86.[19]

The hydrochloride, prepared in acetone by treatment with concentrated hydrochloric acid, was crystallized from methanol–acetone, m.p. 240–242° dec., $[\alpha]^{25}D$ +8.7°.[19]

In one instance, crystallization from benzene gave a different form, possibly non-solvated, which could not be duplicated by seeding or recrystallization. The product, after drying *in vacuo*, had m.p. 160–162°, $[\alpha]^{25}D$ −24.7°. The melting range of a mixture with the form above was intermediate, softening at 155° and melting at 158–159°.[19]

The infrared spectrum in (Nujol mull) had peaks at 3.11 and 5.85 μ and differed in the fingerprint region from that of the low-melting form. The spectrum in solution (CS_2), however, matched the corresponding spectrum of the other form exactly, with peaks at 2.79, 3.0 and 5.88 μ. The hydrochloride prepared from this material melted at 238–240° dec. and did not depress the melting point of a mixture with the original hydrochloride.

3-Esters of 16α-Piperidino-5-pregnen-3β-ol-20-one.—A general esterification procedure for the preparation involved treatment of the alcohol in 4–10 volumes of pyridine with 1–2 parts of acid chloride or anhydride. Thus, 5 g. of 16α-piperidino-5-pregnen-3β-ol-20-one was dissolved in 20 ml. of dry pyridine and treated with 10 ml. of acetic anhydride. The mixture soon clarified and darkened. After standing 2 hours at room temperature, the solution was poured into water. When the excess acetic anhydride had hydrolyzed, the solution was made basic and the product filtered off. Crystallization from ethanol gave 16α-piperidino-5-pregnen-3β-ol-20-one 3-acetate, m.p. 176–178°, $[\alpha]^{25}D$ −22°.[19] The infrared spectrum (CS_2 solution) showed bands at 5.78 μ (ester C=O), 5.88 (C_{20} C=O), 6.02 (Δ^5), and 8.00 (ester C–O–C).

In the same manner, using 1 part of isobutyryl chloride in 10 volumes of pyridine for 8 hours, 16α-piperidino-5-pregnen-3β-ol-20-one 3-isobutyrate was obtained, m.p. 140–141°, $[\alpha]^{25}D$ −12.6°.[19]

Formation of esters with less active acids was incomplete unless the reaction was warmed. Care had to be taken that the heating was only moderate in order to avoid deamination. Thus 16α-piperidino-5-pregnen-3β-ol-20-one was treated with 1 part of veratroyl chloride in 10 parts of pyridine, warmed at 45–53° for 4 hours, and stirred at room temperature overnight. The usual work-up gave 16α-piperidino-5-pregnen-3β-ol-20-one 3-veratrate, m.p. 154–155.5, $[\alpha]^{25}D$ +18.6°.[19] The ultraviolet spectrum (EtOH) showed a minimum at 235 mμ, and maxima of ε 14,050 and ε 7,050 at 261 and 291 mμ. The infrared spectrum (CS_2) showed bands at 5.85 μ (ester and C_{20} C=O), 5.98 (Δ^5), and 13.10 (phenyl).

Deamination of 16α-Piperidino-5-pregnen-3β-ol-20-one.—Five grams of 16-piperidinopregnenolone in 25 ml. of acetic anhydride was refluxed for 2.5 hours, treated with activated carbon (Darco G-60), filtered hot and chilled. The product was collected, washed with methanol and dried; weight 2.54 g., m.p. 175.4–176.9°, λ_{max}^{EtOH} 238 mμ, ε 9400. Recrystallization from isopropyl alcohol gave pure 5,16-pregnadien-3β-ol-20-one acetate, m.p. 176.2–177.2°, λ_{max}^{MeOH} 239, ε 9550.

5,16-Pregnadien-3β-ol-20-one Isobutyrate.—Five grams of 16α-piperidino-5-pregnen-3β-ol-20-one was dissolved in 25 ml. of dry pyridine, treated with 3 g. of isobutyryl chloride and refluxed 20 hr. The dark mixture was evaporated *in vacuo*, and the residue was taken up in benzene, washed with 10% aqueous sodium carbonate and water, and dried over anhydrous magnesium sulfate. Evaporation gave a crystalline residue, wt. 4.5 g., which was crystallized several times from methanol to give the product, m.p. 164–165°, $[\alpha]^{25}D$ −39.1°, λ_{max}^{EtOH} 238, ε 9500.

Anal. Calcd. for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found: C, 77.78; H, 9.40.

16α-(N,N-Dimethylbenzylammonium)-5-pregnen-3β-ol-20-one Iodide.—Four grams of 16α-benzyl-amino-5-pregnen-3β-ol-20-one was dissolved in 25 ml. of benzene and 8 ml. of methanol. The solution was treated with 8 g. of methyl iodide and refluxed 1 hr. To the solution was added 0.4 g. of sodium hydroxide (1 equiv.) and 8 g. of methyl iodide. After refluxing 1 hr., the mixture was allowed to stand overnight at room temperature.

The mixture was filtered to remove a slight amorphous precipitate and evaporated to a residue which was digested in ether to give a crude product, wt. 4.5 g., m.p. 220–230° dec. This was crystallized from acetone–methanol and from methanol to give the product, m.p. 228–230° dec., $[\alpha]^{25}D$ ±0° (95% EtOH); sol. methanol, hot water.

Anal. Calcd. for $C_{36}H_{44}NO_2I$: C, 62.38; H, 7.68; N, 2.43; I, 21.97. Found: C, 62.44; H, 7.96; N, 2.28; I, 21.47.

16α-(N-Methylpiperidinium)-5-pregnen-3β-ol-20-one Iodide.—Four grams of 16α-piperidino-5-pregnen-3β-ol-20-one was suspended in 25 ml. of benzene and 8 ml. of methanol. The mixture was treated with 3.5 ml. of methyl iodide (9.0 g., g., 5.5 equiv.) and refluxed for one hour. The solid dissolved and a precipitate started to form in 20 minutes. The mixture was cooled, and the solid was collected on a filter, washed with benzene and dried *in vacuo;* wt. 3.55 g., m.p. 265–268° dec. Crystallization from methanol–benzene gave material, soluble in alcohol and hot water, with m.p. 271.5–272.5 dec., $[\alpha]^{25}D$ +18.4° (95% EtOH).

Anal. Calcd. for $C_{27}H_{44}NO_2I$: N, 2.59; I, 23.45. Found: N, 2.65; I, 24.15.

16α-Piperidinoallopregnan-3β-ol-20-one.—Two grams of 16α-piperidino-5-pregnen-3β-ol-20-one was dissolved in 50 ml. of glacial acetic acid and treated with 1.5 g. of 10% palladium-on-charcoal. The mixture was shaken with hydrogen at one atmosphere and 25° until absorption stopped (1.15 equiv.). After filtration, the solution was poured into 1 l. of 10% aqueous sodium carbonate, and the crude product was collected on a filter and dried, wt. 1.95 g., m.p. 150–162°. It showed no color with alcoholic *m*-dinitrobenzene in contrast to the starting material. Several crystallizations from ethanol gave the desired product, m.p. 169.5–170.5°, $[\alpha]^{25}D$ +49.6° (95% EtOH).[19]

(19) For analyses, see Table III.

16α-Piperidino-5-pregnene-3β,20-diol.—16α-Piperidino-5-pregnen-3β-ol-20-one (4 g.) was suspended in 50 ml. of methanol containing 2 ml. of water, and to this was added a solution of 4 g. of sodium borohydride in 30 ml. of methanol. The mixture was heated to boiling, and the solution obtained was allowed to stand overnight at room temperature. The mixture was poured into 400 ml. of water and the precipitate collected. The air-dried material was crystallized from isopropyl alcohol, collected and dried, wt. 3.3 g., m.p. 145–175°. The infrared spectrum showed no carbonyl peak.

Five crystallizations from ethanol and methanol gave one epimer, probably 20β-,[20] m.p. 184–186°, $[\alpha]^{25}D$ −97.6°.[19] The hydrochloride had m.p. 292.5–293° dec., $[\alpha]^{25}D$ −43.7°.[19]

The mother liquor gave fractions with lower rotations, but another pure epimer was not isolated.

The methiodide prepared by refluxing in methyl iodide and crystallization from acetone–methanol and methanol, melted at 299–300° dec.

Anal. Calcd. for $C_{27}H_{46}NO_2I$: I, 23.34. Found: I, 23.68.

16α-Piperidino-allopregnane-3β,20-diol.—16α-Piperidino-5-pregnene-3β,20-diol (24 g.) was dissolved in 100 ml. of acetic acid and shaken with 2 g. of platinum oxide under hydrogen in the Parr apparatus for about one hour until absorption ceased (*ca.* 6 lb. pressure decrease). The mixture was filtered and the filtrate was poured into 2 l. of water containing 250 g. of potassium hydroxide. The precipitate which formed was collected and refluxed in 700 ml. of methanol with 12 g. of potassium hydroxide in 50 ml. of water for 1.5 hr. The solution was concentrated and the residue treated with 2 l. of water. This product was collected, dried at 60° *in vacuo* for 3 hours and overnight *in vacuo* but still contained water (wt. 49 g.). In order to dry it the material was dissolved in benzene, separated from a water layer, and the benzene layer concentrated to 150 ml. The product crystallized on standing, and was filtered off and dried at 60° for 30 min., wt. 18.5 g., m.p. 178–179°. The mother liquor gave further crops. Several crystallizations from benzene or acetone gave the desired product, probably 20β,[20] which had m.p. 178–180°, $[\alpha]^{25}D$ −55.2°, and an infrared spectrum (Nujol mull) with peaks at 2.80 and 3.12 μ.[19]

Concentration of an acetone mother liquor gave a second form (possibly 20α),[20] m.p. 185–190°, $[\alpha]^{25}D$ −57.0, differing slightly as expected in the infrared spectrum (Nujol mull) with a peak at 3.12 and a shoulder at 2.96 μ.[19]

The methiodide was prepared in the usual way and crystallized from methanol, m.p. 286–288° dec.

Anal. Calcd. for $C_{27}H_{48}NO_2I$: I, 23.26. Found: I, 23.22.

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(20) *Inter alia, cf.* E. L. Shapiro, D. Gould and E. B. Hershberg, THIS JOURNAL, **77**, 2912 (1955); L. H. Sarett, M. Feurer and K. Folkers, *ibid.*, **73**, 1777 (1951); N. L. Wendler, Huang-Minlon and M. Tishler, *ibid.*, **73**, 3818 (1951).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

Optical Rotatory Dispersion Studies. V.[1] The Effect of Isolated Carbonyl Groups and Double Bonds in the Cholestane Series[2]

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RECEIVED SEPTEMBER 12, 1955

Rotatory dispersion curves of various saturated ketocholestanes are presented and it is demonstrated that this method represents a useful new tool for the location of single carbonyl groups in the steroid molecule. The effect of isolated double bonds on the rotatory dispersion has been examined in the cholestane series.

In earlier papers,[3] it has been demonstrated that certain structural changes in the steroid molecule, particularly those involving carbonyl groups, are amenable to correlation with rotatory dispersion curves. For instance, the Δ^4-3-keto moiety could be recognized in a variety of steroids with or without additional substituents by virtue of certain characteristic features ("maxima" and "minima"[4]) in the dispersion curves. While this represents an observation of considerable theoretical interest, from a practical standpoint it is simpler to recognize a Δ^4-3-ketone through certain ultraviolet and infrared absorption bands. This does not, however, apply to locating *saturated* carbonyl groups in the steroid molecule. Ultraviolet absorption spectra are only of very limited value[5] in this respect while infrared spectra are mainly helpful in differentiating between 5- and 6-membered ring ketones although more subtle correlations have also been attempted.[6]

We should now like to report some observations which suggest that the rotatory dispersion curve can become a very promising adjunct to steroid (and possibly triterpenoid) methodology insofar as the *location of isolated, saturated carbonyl groups* in the molecule is concerned and that structural conclusions appear possible which cannot be made with either the use of ultraviolet or infrared spectroscopy alone.

The present study is limited to the cholestane series and thanks to the generosity of various colleagues[7] it has been possible to determine the effect upon the rotatory dispersion curve of carbonyl groups in all but two of the possible locations in the nucleus. As can be seen from Figs. 1 and 2, the individual curves differ sufficiently so that they can be used for characterization purposes. In some

(1) Paper IV, C. Djerassi and R. Ehrlich, THIS JOURNAL, **78**, 440 (1956).

(2) Supported by a research grant from the Damon Runyon Memorial Fund for Cancer Research. We are indebted to the National Science Foundation for funds covering the purchase of the spectropolarimeter.

(3) (a) C. Djerassi, E. W. Foltz and A. E. Lippman, THIS JOURNAL, **77**, 4350 (1955); (b) E. W. Foltz, A. E. Lippman and C. Djerassi, *ibid.*, **77**, 4359 (1955); (c) A. E. Lippman, E. W. Foltz and C. Djerassi, *ibid.*, **77**, 4364 (1955).

(4) See ref. 3a for definition of terms and general experimental procedure.

(5) O. Schindler and T. Reichstein (*Helv. Chim. Acta*, **37**, 667 (1954)) have pointed out that the low intensity band in the 300 mμ region can sometimes be used to differentiate between 11- and 12-keto steroids, but that even slight structural alterations can affect the spectra to a marked extent.

(6) *Cf.* R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

(7) We are greatly indebted to the following investigators for specimens: D. H. R. Barton, R. C. Cookson, E. J. Corey, L. F. Fieser, W. Klyne, E. Mosettig, T. Reichstein, C. Tamm and R. B. Turner.